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Tushar A Deshmukh
M.Sc Agril. Entomology
Dept. of Agril. Entomology,
Post Graduate Institute,
Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola,
Maharashtra, India

NV Lavhe
Assistant Professor of Agril. Entomology, Section of Agriculture Entomology,
College of Agriculture, Nagpur,
Maharashtra, India

VR Ghadge
M.Sc Agril. Entomology
Dept. of Entomology, Post Graduate Institute, Akola,
Maharashtra, India

AD Nichal
M.Sc Agronomy
Dept. of Agronomy,
Post Graduate Institute,
Akola, Maharashtra, India

Corresponding Author:
Tushar A Deshmukh
M.Sc Agril. Entomology
Dept. of Agril. Entomology,
Post Graduate Institute,
Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola,
Maharashtra, India

Reproductive potential of some local entomopathogenic nematode isolates from different cropping system on *Helicoverpa armigera* (Hub.)

Tushar A Deshmukh, NV Lavhe, VR Ghadge and AD Nichal

Abstract
The survey of EPN from different cropping systems revealed that the presence of EPN was found prominent in case of sorghum, soybean, chilli and okra cropping systems. The samples sent for identification confirmed EPN species is *Heterorhabditids indica* in Akola vicinity. *H. armigera* were exposed to 25, 50, 75, 100, 200 IJs /larvae concentration of each EPN isolates to test reproductive potential EPN isolates. Nematodes were harvested using White Traps. Highest multiplication of entomopathogenic nematodes was also recorded in 3rd instar larvae when infected with EPN isolates from soybean and sorghum crop ecosystems, respectively. The maximum number of Infective Juveniles (IJs) was harvested from 3rd larval instar which was infected with 200 IJs/larvae concentration.

Keywords: Entomopathogenic, nematodes, *Helicoverpa armigera*, instar, reproduction

Introduction
Okra [*Abelmoschus esculentus* (L.) Moench] is an annual herbaceous plant, native of tropical Pesticides have harmful effects on environment therefore alternative control strategies are necessary for their management due to increasing concern over human safety and environment (Gaugler, 1988; Villani and Wright, 1988) [7, 16]. Every year farmers spend $300 million on insecticides, of which 80% applied for control of chewing insects (Rao, 2007) [15]. It has threatened biocontrol agents such as parasites and predators. The risk of using insecticides varies in several ways depending upon application coverage (Cilgi *et al*., 1988) [4], their exposure (Kennedy, 1988) [2], as well as upon the intrinsic toxicity of chemicals employed (Hassan, 1987) [8].

Nematodes that naturally occur in the soil can generally be divided into two groups: beneficial (which include EPNs) and non-beneficial (plant parasitic) nematodes (Kaya and Gaugler, 1993; Bird & Bird, 2001) [10, 2]. They have the ability to search for the hosts and relatively safe to non-target organisms and vertebrates (Gaugler, 1981; Poinar, 1983) [6, 14]. The progeny nematodes develop through four juvenile stages to the adult. Depending on the available resources one or more generations may occur within the host cadaver and a large number of infective juveniles are eventually released into environment to infect other hosts and continue their life cycle (Kaya and Gaugler, 1993) [10]. They also have the ability to persist two to three weeks under field conditions (Gaugler, 1988; Kaya *et al.*, 1993; Burnell & Stock, 2000) [6, 10, 3]. EPNs from the genera *Steinernema* and *Heterorhabditis* have been identified as promising biological control agents for inundative biological control of a vast array of economically important insect pests of a wide range of agricultural and horticultural crops (Ehlers, 1996; Hazir *et al.*, 2003) [5, 9].

Materials and method
Collection and rearing of test insect
Healthy larvae of *H. armigera* were collected from field and reared in the laboratory of Entomology Department. They were fed with artificial diet. Rearing procedures for *H. armigera* as prescribed *by* Armes *et al*. (1996) [1] were followed. As far as *C. cephalonica* is concerned the supply of larvae were made by the Bio-control laboratory of the Entomology Department. The 3rd instar larvae of these insects were used as host for EPN in the present studies.

Installation of bait traps in crop soils

A bait trap consisted of a small plastic bottle having perforated lid. About 8-10 full grown *Corcyra* larvae were placed in the bottle and subsequently filled with moist soil near the root zone of the crop. These bait traps were placed below 15 cm soil depth near the root zone in slanting position at 45^0 angle. About 284 such traps were baited in 23 cropping systems at different locations in Akola vicinity and labelled properly. After 10-12 days these bait traps were taken out from the respective cropping systems and inspected for the larval mortality.

Mounting of dead larvae and isolation of EPN

The dead larvae form the bait traps were separated out and mounted on White traps for separation of EPN in vivo production uses a White trap (White 1927). A White trap consisted of a glass petriplate (Make - Duran 150 X 25 capacity) with a small glass petriplate (Make - Borosil 60 X 20 cap.) placed inverted position and filled with double distilled water inside the bigger size petriplate. A piece of filter paper was placed on the small petriplate in such a way that a part of the paper will remain embedded in water. The dead larvae in the baited traps from different cropping systems were mounted on the filter paper and observed for release of EPN in the surrounding water after 10 to 12 days. The larvae placed on mount were also inspected for development of nematodes under cuticle using Stereo zoom binocular microscope for emergence of nematodes. Each white traps were labelled properly with marker pen for different cropping systems. Investigations on killing of larvae in bait traps revealed occurrence of EPN in Sorghum, soybean, chilli and okra cropping systems. The reason for killing *C. cephalonica* larvae in other cropping systems was not the EPN. Hence for isolation and other studies the EPN isolates from these four cropping systems were taken. From these white traps 50 ml nematode suspensions in double distilled water were prepared and used for further studies.

Counting of EPN in suspension

Initially 50 ml EPN suspension collected in White traps from sorghum, soybean, chilli and okra cropping systems were taken. The infective juvenile (IJ) count was taken by counting the number of IJs per 100 µl of EPN isolate from the respective cropping systems. For this purpose 100 µl of suspension was measured using a micro pipette and poured on a sterilized glass slide. It was further observed through Stereo zoom binocular microscope for number of IJs. Accordingly, the suspensions were standardized for EPN IJs and used for further experimentation.

Multiplication and culturing of EPN

In order to get pure culture of the respective EPN isolates they were re-infected to the larvae of *C. cephalonica* at ambient laboratory conditions. Initially a measured amount of suspension with standard count of IJs/100 µl from the respective EPN isolates from soybean, sorghum, okra, and chilli cropping systems were taken and 10 larvae of *C. cephalonica* were inoculated by direct contact method. After the interval of 10-12 days the nematode suspension with infective juveniles from all four isolates were collected and re-infected to fresh *C. cephalonica* larvae and this process of inoculation and re-infection of nematode suspension to larvae of *C. cephalonica* repeated until the pure culture of nematode populations with infective juveniles were obtained. These pure cultures were used for preparation of different doses / concentration for treatments for further studies. The standard IJ counts were prepared by adopting serial dilution method using double distilled water.

Reproduction of EPNs on *H. armigera*

In this experiment the 3rd instar larva of *H. armigera* were exposed to 25, 50, 75, 100, 200 IJs /larvae concentration of each EPN isolates (Yadav and Lalramliana, 2012) [18] in separate petri dishes and total number of IJs produced /larva up to a period of 20 days were counted. The nematode infected dead larvae were removed from dishes, rinsed in sterilized distilled water and transferred individually on to white trap for their emergence from the body (White, 1927). Larvae were collected daily for up to a period of 20 days till the emergence of IJs will stop from insect cadavers and total number of IJs /larva were determined. There were four treatments (EPN isolates) and a control (Water) replicated four times under CRD design.

Results

The reproduction / multiplicity of various EPN isolates from soybean, sorghum, chilli and okra on 3rd instar larvae of *Helicoverpa armigera* with different concentrations were studied in laboratory under ambient conditions of temperature and humidity and the results have been depicted in following tables under.

Table 1: Reproduction of EPN isolates with 25IJs concentration on *Helicoverpa armigera* larvae

Sr. No.	Treatments	EPN count (25IJs/100 µl) obtained				
		R I	R II	R III	R IV	MEAN
1	Sorghum	131	135	134	140	135.00
2	Chilli	125	123	129	131	127.00
3	Okra	128	131	135	132	131.50
4	Soybean	123	117	120	125	121.25
5	Control	0	0	0	0	0.00
	F-test	Sig		CD at 5%	4.66	
	SE (m)	1.54		CV (%)	3.00	

Table 2: Reproduction of EPN isolates with 50IJs concentration on *Helicoverpa armigera* larvae

Sr. No.	Treatments	EPN count (50IJs/100 µl) obtained				
		RI	RII	RIII	RIV	MEAN
1	Sorghum	142	147	149	151	147.25
2	Chilli	139	135	140	142	139.00
3	Okra	140	138	146	149	143.25
4	Soybean	141	148	138	150	144.25
5	Control	0	0	0	0	0
	F-test	Sig		CD at 5%	6.10	
	SE (m)	2.02		CV (%)	3.53	

Table 3: Reproduction of EPN isolates with 75IJs concentration on *Helicoverpa armigera* larvae

Sr. No.	Treatments	EPN count (75IJs/100 µl) obtained				
		RI	RII	RIII	RIV	MEAN
1	Sorghum	162	152	153	159	156.50
2	Chilli	139	143	145	147	143.50
3	Okra	149	152	156	159	154.00
4	Soybean	151	148	159	161	154.75
5	Control	0	0	0	0	0
	F-test	Sig		CD at 5%	6.49	
	SE (m)	2.15		CV (%)	3.54	

Table 4: Reproduction of EPN isolates with 100IJs concentration on *Helicoverpa armigera* larvae

Sr. No.	Treatments	EPN count (100IJs/100 µl) obtained				
		RI	RII	RIII	RIV	MEAN
1	Sorghum	169	165	172	175	170.25
2	Chilli	158	155	163	165	160.25
3	Okra	163	168	171	178	170.00
4	Soybean	165	171	169	174	169.75
5	Control	0	0	0	0	0
	F-test	Sig		CD at 5%	6.49	
	SE (m)	2.15		CV (%)	3.21	

Table 5: Reproduction of EPN isolates with 200IJs concentration on *Helicoverpa armigera* larvae

Sr. No.	Treatments	EPN count (200IJs/100 µl) obtained				
		RI	RII	RIII	RIV	MEAN
1	Sorghum	190	185	179	192	186.50
2	Chilli	163	175	178	181	174.25
3	Okra	182	187	188	185	185.50
4	Soybean	178	182	189	192	185.25
5	Control	0	0	0	0	0
	F-test	Sig		CD at 5%	8.08	
	SE (m)	2.68		CV (%)	3.66	

Heterorhabditids indica from different cropping system showed 100 % mortality in 3rd larval instars of *Helicoverpa armigera* (Hub.) after 4 days exposure time. EPN multiplication in larvae of *H. armigera* (Hub.) Was examined by comparing the number of nematodes in each larval instar and number of nematode counted up to 20 days. The result depicted from Table 1,2,3,4 and Table 5 revealed that the highest reproduction and recovery of infective juveniles obtained from 3rd instar were when infected with 200IJs/larva as compared to other concentrations i.e. 25, 50, 75,100 IJs. Results were observed in a study that among the treatments of EPN isolates obtained from different cropping systems-okra, soybean, sorghum, chilli maximum nematodes were harvested from sorghum EPN isolates on *H. armigera* (Hub.)

Discussion

Reproduction and recycling of EPN strain in host plays an important role in their persistence in soil, infectivity and overall effectiveness in pest control. A prior knowledge about reproduction and recovery of the EPN is considered important in determining the time and dose of subsequent application in field. As far as reproduction and recovery of IJs from various EPN isolates in the present investigations is concern, the data on reproduction suggest that following treatment inoculation, all the tested EPN isolates from the four cropping systems were able to infect and propagate within the body of the host insects viz., *Helicoverpa armigera* used in the present investigations. The highest recovery of IJs was obtained from 3th instar larvae at a concentration of 200 IJs/100µl in all the two insects under study. These results match the assumptions by Koppenhofer and Kaya (1995) [12] who assumed that the final EPNs population varies with the number of IJs inoculated. Although, they could not resembling the results of the studies conducted by Meshram (2015) [13] who obtained highest recovery of IJs from the 5th instar larvae of *Helicoverpa armigera*, *Argyrogramma signata* (green semilooper) and *Corcyra cephalonica* at fixed treatment concentration of 50 IJs/100µl of EPN suspension isolated from soybean and mungbean ecosystems. Observations on the similar line were also reported by Yadav and Lalramliana (2012) [18] on indigenous EPNs from Meghalaya against Taro leaf beetle *Aplosonyx chalybaeus* (Hope).

The reproduction and multiplication studies revealed that recovery of EPN Infective Juveniles (IJs) was highest in larvae of *H. armigera* when infected with 200 IJs/100µl concentration sorghum EPN isolates from okra and chilli, soybean and sorghum cropping systems with concentrations 25, 50, 75,100 and 200IJs, respectively. From the studies conducted it can be concluded that EPN *Heterorhabditids indica* found in the soils of this region shown good potential as pest management agent against polyphagous pest like *H. armigera*.

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